THE BOTTLE-ANIMALCULE, FOLLICULINA; ŒCOLOGICAL NOTES.

E. A. ANDREWS.

The following observations upon the œcology of the marine protozoan *Folliculina*, known to some microscopists as the Bottle-animalcule, were made in connection with a study of its life history and method of forming its bottle-shaped case that will be published elsewhere.

In the summer of 1912, and again in 1913, vast numbers of these interesting protozoa and their cases were found on aquatic plants in the Severn River, which is a brackish side branch of the Chesapeake Bay.

The wide distribution of this highly specialized marine *Stentor* is indicated in the following brief outline of the history of our knowledge of it. Discovered by O. F. Müller in 1781 on the Danish coast, it was next studied on the Norwegian coast in 1858 by Claparde and Lachman and on the British coast by Strethill Wright; at Wismar by Stein in 1861; near Kiel by Möbius in 1865; again on the British coast by Saville Kent in 1870–1879; near Naples by Geza Entz in 1884 and in the same year by A. Gruber at Genoa.

Recently, 1910–1913, Carl Dons had described various *Folliculinas* from Norway, Spitzbergen and Iceland as well as the Adriatic, while Laachmann has found them in West Australia, Sumatra and the Antarctic.

The only records of its occurence in American waters is that of Leidy, who in 1859 found it at Newport, R.I., on the Atlantic coast of the United States while dredging with Col. Powel, and that of Ryder, who in 1880 described it as found in quantities on oyster shells from the west coast of the Chesapeake Bay.

In the sea *Folliculina* has been found living attached to various red and brown algæ and other plants as well as upon stones, the shells of molluscs, and annelids and the tests of hydroids, bryozoa and tunicates from shallow shore waters down to depths

of four hundred meters. A few were taken on the surface of the water.

There are, however, records of its occurrence in fresh waters; thus Saville Kent in England described *Folliculina boltoni* from *Anacharis* and other plants as living in fresh water, and Barrett found it in the fresh waters of the Thames; while the form called *Ascobius lentus* by Henneguey in France found in flowing fresh water in the botanic garden at Montpelier on the leaves of *Aponogeton dystachium* seems to be a kind of *Folliculina*.

The *Folliculina* found in the Severn are no doubt *Folliculina ampulla* in the wide sense used by Stein and by Möbius and resemble most the forms described by Strethill Wright, but in the light of the revision of the group by Carl Dons the specific determination of these Severn forms is deferred till a more complete comprehension of their life history furnish a better

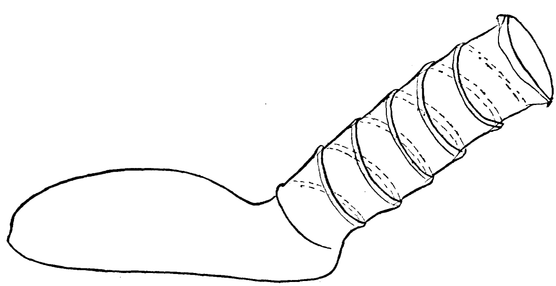

FIG. 1. Side view of case made by *Folliculina*. Camera lucida. × 233.

basis than the mere form of secreted case the animal inhabits. Both *Folliculina* and *Parafolliculina* forms in the sense used by Dons occur here; but the character of nucleus and existence of micronucleus necessitates much modification in his classifications. It may well be that not only *Freia* and *Lagotia* but also *Ascobius* are synonyms of *Folluculina* and that many of the species are but phases in a protean life cycle.

The most usual form of dwelling inhabited by *Folliculina* in the Severn is shown in Fig. 1 enlarged 233 diameters. For convenience of description it might be regarded as made up of a sac and a tube, both together constituting the case. The sac is the part of the case attached to some foreign object and the tube is the free part. The sac is wider from side to side than it is

deep, since its attached face is flattened out against the support, while the free face or back is very convex. The posterior blind end is bluntly rounded and affords attachment for the foot end of the animal. The tube rises at about 45 degrees from the axis of the sac or the plane of attachment, and ends in a freely open mouth without valves but with a reflexed lip. As a rule about six turns of a spiral-ridge pass about the tube, but the number is not fixed. The spiral seen from the right side, Fig. 1, passes toward the top of the tube over the dorsal side. The nature and mode of formation of the case and the spiral of the tube will be described elsewhere.

Areal and Depth Distribution in the Severn.

First seen on the leaves of *Elodea* (*Anacharis*), the cases or bottles of these animals seemed to the eye mere dark lines $\frac{1}{2}$ mm. long that might be excrement of some small snail lying abundantly on the leaf amidst sediment.

But the microscope reveals marvelous greenish, transparent, bottle-like cases containing a clear, but blue-green animal that shyly withdraws till no longer disturbed and then may, from time to time protrude from the mouth of the bottle as a slender thread divided like a snake's tongue into two forks, which are held curved or else bent about and waved till all suddenly vanishes as a mere lump within the bottle. The animal might be compared to a miniature fork with long bent arms all made of glass but more pliant and elastic than rubber.

The cases with or without the contained animal were found on the leaves of several plants, chiefly *Elodea* and *Potamogeton* growing upon the bottom of this decidedly saline water, and also upon floating leaves and stems of these plants in the open river as well as on the sides of a boat and on wood. These plants form zones along the shores of most of the side branches of the river, known as "creeks"; the *Potamogeton* zone is nearer shore and grows in water one to two feet deep; the *Elodea* appears as a blue-green zone some twelve feet wide in water two to six feet deep, at high tide (the water falling one to two feet). *Folliculina* was taken from "Back Creek" below Annapolis up beyond the "White Sands," some nine miles, in side creeks on both shores.

The *Elodea* generally grows in a dense continuous plantation the crowded tops almost touching, so that in a square foot there is at least one plant with some fifty leaves on its upper four inches where the *Folliculina* tubes abound; on an average each leaf has five to ten tubes; many have much greater numbers. Allowing 250 tubes to the square foot, which is a minimum estimate for the more sparsely populated regions, and estimating the length of the *Elodea* zone as twenty miles and its width as only 4 feet, since the *Folliculina* are most abundant in water 3–4 feet deep and not in the upper and lower edges of the *Elodea* zone, we would have some one hundred million of these miniature dwellings along the Severn. They were found also in abundance at the head of Whitehall river which opens independently into the Chesapeake just to the north of the Severn, and as they are probably widely distributed along the branches of the Chesapeake we may regard *Folliculina* as a numerically important member of the summer fauna of this large body of salt water.

The following experiments throw light upon the distribution of the tubes of *Folliculina* in various depths of water. Late in July with water at 28° C. strips of smoothly sawed pine wood 35 mm. wide and 9 mm. thick were stuck upright in the mud in a row across the *Elodea* zone and left six days. On all strips in from two feet to five feet depth of water many tubes of *Folliculina* were attached, but they were absent from the bottom for some eight to twenty inches above the mud and from the top some nine inches below high tide line. The stick nearest shore in two feet water had a sparse population, perhaps 100 tubes to the square inch, while the sticks farther out showed in places as many as 400 per sq. inch. In these latter the tubes were often aggregated in clusters, one containing as many as 73 tubes, while in the more shallow water the tubes were scattered and more irregular in distribution. To see if the tubes would be made in depths beyond the *Elodea* zone the same strips of wood were tied together in a row, end to end, and anchored in the middle of the mouth of a creek where there was no plant life on the bottom, the water being 15 feet deep, and the *Elodea* zone several hundred feet distant. After a submergence of three days and four nights all the four sections of the compound strip

were well set with new tubes (the wood was dried and sand-papered before this experiment). The lowermost section of wood reaching from the bottom up six feet showed tubes about twenty to the square inch with but few in small clusters, but the lowest two feet contained fewer and the bottom few inches, none. The second section five feet long had as many, if not more, tubes than the lowest section and more clusters, one containing as many as 20 tubes. The third section was three feet long and reached up above the surface of the water. The tubes on it were as on the section below it but became very sparse near the surface of the water. The fourth section floated out diagonally in the water but was only partly submerged and it contained but few tubes.

In brief the tubes had been made on the strips of wood from near the surface to within a few inches of the bottom, when there were no plants near from which the animals might have migrated.

It is thus evident that *Folliculina* occurs in greater depths than the zones of vegetation in these creeks, but normally it can find solids for attachment almost only within these zones, since the region is without stones or rocks and with teredo fauna that rapidly removes submerged woodwork.

Folliculina is thus forced into association in depths with *Elodea* and the few other flowering plants that follow the shore.

Seasonal Distribution.

These waters undergo great seasonal changes; in the high temperatures of summer the water is turbid with microscopic life, but more clear in winter.

The grosser life of the plant zones along shore also rises to a maximum in the summer and in the winter disappears down to the bottom of the water where only the roots and pieces of stems remain to revive in the following spring.

Folliculina is thus forced into seasonal periods of abundance and disappearance; but while it is abundant only with the growth of the plants it has a much shorter period than they, appearing in the summer after the plants are well started and vanishing long before the plant zone dies down.

In 1912 no live *Folliculina* could be found after September 8,

though in aquaria they lived till the 27th and in a warm room till November 11, though in a faded and apparently starving state. In 1913 there was a sudden disappearance of most all live *Folliculina*, leaving chiefly empty tubes, after a sudden drop of temperature from 97° F. to 60° F. with rain. No live ones could be found after September 5 after cold rain, though found September 1 in a very few examples after much search.

By September 22 with the water at 83° F. and air at 80° F. no tubes even could be found except in places where the plant had not recently grown enough to shed the leaves to which the *Folliculina* tubes had been long attached.

Such empty tubes remaining upon old leaves that have not yet fallen off, illustrates one phase of the correlation between *Folliculina* and the growth of the plants.

Followed from spring onward the distribution of the living *Folliculina* is nicely adjusted to the growth of the plants, as will be seen from the following.

May 17, 1913, no *Folliculina* had yet appeared, but the *Elodea* zone denuded all winter had begun to be repopulated with new plants shooting up three inches high, apparently from old bits of stem buried in the mud. These plants were then perfectly clean and bright with no inhabitants save a few minute young snails. Some of the cells of the *Elodea*, however, were already dead here and there, apparently from some parasite; but this was not connected with *Folliculina* that at present was in some unknown state. The *Potamogeton* zone showed stalks eighteen inches high with some few flower buds far from the surface of the water.

By June first the *Potamogeton* had flowers near the surface and the *Elodea* was 12 to 15 inches high and no longer entirely clean but with the older lower leaves covered with a flocculent deposit and supporting many colonial vorticellids seen to the eye as contractile tufts on single stalks. Two weeks later, the *Elodea* was two feet high with flowers on stems four to six inches above leaves and some open flowers at surface. The *Potamogeton* now was three feet high with flowers at the surface of water. But all the leaves were covered with dirt and no *Follicullina* was found. On the 27th, *Folliculina* was found as black

patches 1 to 2 mm. in diameter here and there on the leaves of *Elodea* in water two to six feet deep, otherwise the leaves were clean. These patches or colonies of *Folliculina* were only one to the leaf, either at the tip or at the edge of the upper surface. The origin of these first tubes was not determined. Aquaria that contained *Folliculina* in the fall still contained the empty tubes at this season but no revival of *Folliculina* was found in the aquaria even though the aquaria were kept all the summer.

Whether the *Folliculina* passes the winter in some form in the mud to develop when the conditions are right or whether it migrates into the river each season and becomes attached when the growth of the plants, the temperature and food conditions permit, is not known. The fauna of the Bay and river is markedly increased by migrants; not only are there vast shoals of jelly-fish (*Chrysaora* form of *Dactylometra*) and of ctenophores that come and go with menhaden and other fish but the plants that grow along the shores become the support for bacteria, diatoms, protozoa, anemones, polyzoa, tunicates and nudibranchs, that disappear in the fall and seem to have arrived as plankton and to have multiplied or grown in the temporary forcing house that this body of water forms in the summer. With the water gradually becoming opaque green from the multiplication of the microscopic biota and the temperature running up to 27° to 30° C. in July when the air is 28° to 29° C. *Folliculina* with many other organisms lives in ideal conditions for rapid multiplication, so that a few immigrants from the sea might produce the entire population that dies out at the oncoming of cool weather and the great changes in the gross and microscopic fauna and flora of the shores. Thus *Folliculina* and other marine animals become temporarily associated in a summer community dependent upon the zones of essentially fresh water plants, *Elodea* and *Potamogeton*.

The Chesapeake and its branches is a typical "drowned river" and we find here a tendency toward a gradual capture of the fresh-water biota by the incoming marine biota. Thus the marine blue-crab with many sea fish migrates over the line between the fresh and salt waters and at times becomes an important factor in the œcology of fresh and of marine organisms as well as of the dwellers on the transition line.

In the general process of capture of fresh by marine biota, may be included the temporary utilization of the fresh water plants, *Elodea*, *Potamogeton*, etc., by the summer immigrants from the sea. The swarms of *Folliculina* settling in the *Elodea* zone is but one illustration of the push from the sea inland.

The waters of the Severn support a thousand acres of natural oyster bars and are thus marine rather than freshwaters. The water in this river as published in 1912 by the Shell Fish Commissioners of Maryland varied from 1.0036, 1.0048 in July to 1.011 in December and 1.0096 in March; the gravity of the ocean being from 1.025 to 1.027. It should be emphasized, however, that each creek has some little spring or stream of fresh water entering its head as representative of the former head-waters of a side branch of the original fresh stream and where this water meets the saline tide waters there is an oscillation of conditions from the densities of high tides in dry seasons to the extreme freshness of low tides and in-pour of torrents of muddy water after rains. It is between these oscillation areas on the one hand and the more continuously saline depths on the other that the *Elodea* zone is most pronounced.

By July 4, *Folliculina* was very abundant over the leaves of *Elodea* in small scattered colonies of each a few individuals; by the 13th, exceedingly abundant on most all the leaves of the top two to four inches of *Elodea* in two to four feet depths in a zone 12 feet wide; they also formed a black area a few inches wide close to the surface of the water along the sides of a floating boat, twenty feet from the *Elodea* zone.

With the progressive growth of *Elodea* the upper leaves are the only ones upon which live *Folliculina* is found while the older leaves are thick-set with empty tubes. As the plant grows upward the shaded old leaves macerate and disappear but the tubes fall off to the bottom and may be got in great numbers from the mud as well as from the macerated dead leaves. In August, *Elodea* may be four feet high, but only the upper foot has leaves, and on these the dense settlements of *Folliculina* are generally a few inches from the top. Examination of the leaves shows the youngest Folliculinas on the uppermost or youngest leaves and so on down. Thus during the growing season there

is a constant succession of new tubes formed above on the more recent leaves while lower down the old tubes are doomed to be dropped off from the macreated leaves to the bottom where they long resist decay. Both the gradual spread of *Folliculina* tubes from the first seen on the tips of the leaves over the entire expanse of upper leaves and the secondary migration from these leaves ever upward to other leaves as they grow out from the terminal buds might be accounted for on the hypothesis that the entire occurrence of *Folliculina* in these waters is the result of inoculation each spring with new individuals from outside the region and the rapid multiplication, spread and autumnal destruction, under seasonal changes of environment.

Sessile and Motile Forms.

Direct observations in aquaria showed that many of the above facts as to the attachment and occurrence of tubes were due to the existence and behavior of free swimming motile states of this animal. When brought into dishes of various kinds these motile forms soon swarm out from the tubes and construct tubes attached to the sides of the dish. Under the microscope, some of the *Folliculina* were seen to emerge from the tubes and to eventually swim free in the water. With the naked eye the free swimmers were followed in the aquarium till they became attached to its sides. When the *Elodea* was collected in the evening free swimmers were found the next morning and made new tubes during the day. In this way as many as four successive crops of newly attaching *Folliculina* were obtained in successive days from one collection of *Elodea*. The newly settled animals lived probably at least two weeks though the longest observation of individuals was nine days.

While most of the new swimmers came from old tubes on the *Elodea* there was good evidence that the new tubes formed in captivity gave rise to swimming forms. When collected early in the morning free forms were found before noon and new sessile forms were made in the afternoon. While the temperature seemed to influence the times, in general no swimmers were liberated in the night but began about seven or eight in the morning and swam two to several hours; about noon the sessile

form was begun with the making of cases which were completed in from four to eight hours; in the evening one to several hours, often, were taken to perfect the organization of the sessile form in its new case.

Within the twenty-four hours the entire cycle from sessile to new sessile form was completed, and in extreme cases, only six hours were necessary.

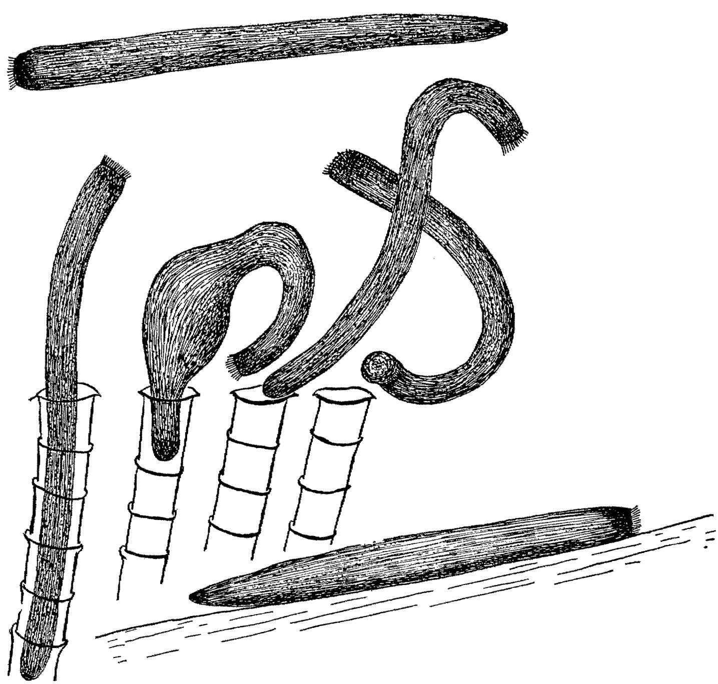

FIG. 2. Sketch of motile form emerging from tube. Four successive stages of one individual in row from left to right with same individual above swimming free and later, below, gliding over surface of leaf.

In nature then it is probable that the *Folliculina* on leaves of *Elodea* give off swarms of motile forms that find the new upper leaves, as well as floating leaves and objects in the deep waters also as shown in the above experiments, and quickly settle down to remain in new tubes till these leaves in turn become unfavorable from accumulations of dirt and from shading by overhanging leaves. Then there is new migration upward and so on as long as food, temperature and other conditions allow.

Failure to collect such motile forms in the open by dragging nets over and through the *Elodea* was in part due to the lateness of the season, August 19–20, and in part to the difficulty of retaining ciliated cylinders one tenth to one twentieth of a millimeter in diameter in a bolting cloth of one tenth millimeter mesh.

The motile forms must be very abundant at times in all these waters and through the summer floating branches of *Potamogeton* and of *Elodea* taken in the middle of the river are more thickly covered with tubes than those growing along shore, as if they had been colonized while floating. Far swimming motile forms carried by the currents may be the first settlers in the spring that so rapidly take possession of all the *Elodea* zones. Such motile forms must be a considerable addition to the plankton and may fall a prey to the schools of young menhaden that frequent the river and its branches, feeding over and near the *Elodea* zone as well as farther from shore.

That the sessile forms have some enemies is seen in the utilization of their cases by the larvæ of aquatic insects that bite them off and from them construct sinuous tubes in which they lie stretched out over the surface of the leaves, with green contents in their digestive tracts strongly suggesting that they not only destroy the cases of *Folliculina* but eat the contained animals; and one larva was seen to bite off and chew up a *Folliculina* recently settled on filter paper.

The distribution of the *Folliculina* cases over the leaves being due to the behavior of the motile forms, two questions call for answer; why are the upper leaves selected and why are the cases placed in groups or aggregates? The following observations on the actions of the motile forms aid in answering these questions.

Behavior of Motile Forms.

The motile forms seen emerging from tubes, Fig. 2, are long cylinders abruptly truncated at the anterior end and bluntly pointed at the posterior end. They change form by local contractions, bending the part outside the tube in various directions and swelling and contracting in diameter in some regions at expense of others. The figure represents one in successive poses from left to right, finally leaving the tube. After some minutes,

of thus, as it were, feeling about, the *Folliculina* swims away from the tube, as in the upper part of Fig. 1 and then resembles a spirostomum. It may sooner or later settle upon leaf or stem and glide about, as in the bottom of Fig. 1, resembling then a planarian in motion. While very often an elongated cylinder it changes in swimming or gliding to various shapes, bending and contracting, may often assume a bottle form from the swelling of the posterior end, become flattened or take the form of a spheroid. The body is generally dark blue-green with the anterior part frequently much darker than the rest since a short region back of the truncated end is sharply set off as almost black and very opaque. The posterior end is frequently dark also.

The form assumed is often that of some species of *Stentor* in motion, and study of compressed living as well as sectioned material shows that they have the organization of *Stentor cœruleus* as described by Schroeder and verified by me on the same form for comparison; but they present certain marked simplifications at the anterior end and a complexity at the posterior end that will be elsewhere described.

These swimming forms show a great diversity in size as well as in form and in color density. Often they measure about one half millimeter in length.

The locomotion seems due to the longitudinal rows of cilia that cover the body and not to the abbreviated spiral of membranellæ at the anterior end. They swim forward with rotation, the right side coming up and over to the left, probably four to five times a second. The speed is two to four millimeters per second, but it changes. After long continuous stretches of motion it may advance, with interruption in jerks, as if the cilia stopped and resumed. Frequently the direction is changed and the phenomena of reversal and advance in another direction on approaching the edge of the water seem like those of *Paramœcium*. While the trajectory appears straight, often, it may again be a long sinuous curve and often a spiral. After stopping the animal may turn off and progress at an angle with its former course, as above stated, or it may move backwards nearly on its trail. It may reverse, spin on its transverse axis and then resume the old direction.

In a very small drop of water the *Folliculina* may go back and forth again and again like a trolley car, reversing at each end of its short trip. One individual with a fixed black spot on the anterior end was seen to go forward and backward and to spin about its transverse axis without revolving about its long axis.

Responses to Light.

It is very striking to see the completeness with which light determines the distribution of the swimming *Folliculina* in aquaria. If in an opaque dish the tubes are all made on the side most illuminated, towards the window in a room, or if in a square glass vessel all tend to crowd to the angle nearest the strongest light window. Watching the swimmers we see that they go rapidly toward the light and only turn back upon striking an obstacle such as the side of the dish when they may swim away for a distance soon to return and eventually to remain nearest to the light. The new tubes are then built along the side toward the light and most all are very near or at the surface of the water unless some irregular reflection determines the formation of two bands, one near the surface and one deeper down, but while the densest crowd is close to the surface, tubes are made an inch and a half below the surface in a scattered arrangement. New tubes formed on strips of paper and glass slides at the side of the dish were removable for study and preservation. When the aquarium is placed on the floor and lighted largely from above, many tubes are formed on the surface of the water, either on floating objects as cover glasses, pieces of paper, leaves, etc., or merely on the surface film. In this way new tubes with the contained animals were obtained free from all solid substratum and excellent for sections and other manipulation.

By placing an opaque paper with central hole over the dish containing the *Elodea* and old *Folliculina* the new formed swimmers were concentrated to the center of the dish and could be taken out with the pipette or later taken up as floating colonies away from the sides of the vessel and in a limited area.

While most all of the swimmers collect toward the light some may form tubes on the sides of the vessel away from the light, but these are much in the minority. In a watch-glass the swimmers go at once to the side whence the light comes.

Responses to Solids.

On emerging from the old tube the swimmer may not at once swim toward the light but glide along on the leaves of *Elodea;* such rapidly gliding *Folliculina* suggest planarian locomotion; they seem investigating the surface by bending in and about amidst diatoms and detritus and crawling over complex surfaces as if attracted by contact. After swimming to the sides of a vessel the *Folliculina* is markedly affected by contact, often at once ceasing to swim and beginning to glide along the surface of whatever nature it may be; but this adjustment to the surface does not necessarily dominate for long, as the animal may suddenly swim free again for a short distance away from the surface to return again. In this way it is a gradual process of coming and going that finally results in the arrest of all motion on the surface of future attachment of the new cases. When arrested by a surface the animal abruptly changes form, frequently becoming spherical or bottle-shaped. In some cases the body is markedly flattened out against the surface and the form may be that of a pocket flask some two times as wide as thick. In gliding as in swimming, *Folliculina* may advance in jerks, or abrupt changes at very short intervals.

While the free swimmer comes to rest and adheres to the surfaces of leaves, paper, glass and porcelain as well as rough wood there are some surfaces that are less available as indicated by the few tubes formed on the resinous parts of the pine strips above referred to, and by the failure of the following experiment.

August 5, in a region where *Elodea* was nearly black with tubes of *Folliculina* and many were also present upon *Potamogeton*, plates of wax "foundation" as used by apiarists were suspended in wooden frames so as to float just above the *Elodea* in five to six feet of water. In six days but three or four very young tubes were found on both surfaces of the two sheets of wax, though the tubes were scattered over the narrow wood frames. Ten days later the tubes were more numerous on the wood, but only a dozen were on the wax.

Though these experiments were tried too late in the season to get a marked attachment of free swimmers yet it shows that the small area of wood was more effective than the greatly larger

area of wax, though the latter presented many facets and edges. To this evidence of discrimination in the surfaces settled on should be added the fact that the tubes were not made on the lowermost inches of strips of wood, even when there was no plant present to shade the bottom (as above described) and few tubes were attached to the more resinous parts of the wood, so that the swimming *Folliculina* would seem to react to various stimuli.

Again in the experiments with strips of wood the tubes fastened to the wood both in shallow and in deep water were noticeably orientated with the mouth of the tube upward toward the surface and the tubes were prevailingly situated lengthwise in the furrows of the wood caused by the saw and grain.

On the stems of *Potamogeton* also the tubes stand lengthwise and generally with the mouths upward. On leaves the tubes are generally more abundant upon the upper than the lower surfaces and often are crowded toward the edges.

The above response to light would lead to the successive colonization of upper leaves in the *Elodea* zone during the season and would explain the common orientation of the tubes with mouth upward.

The responses to solids would lead to the rapid utilization of most solids as basis for attachment of tubes. But evidently some other factors must be concerned in the choice of certain surfaces, or refusal of some, and in the selection of certain sites, as grooves and especially the crowding into aggregates as if aware of one another's existence.

Aggregation.

Though isolated cases or tubes of *Folliculina* are common on leaves as well as upon material experimentally supplied in the open and in the laboratory for the attachment of the swimming forms, yet it is very noticeable that the great majority of *Folliculina* tubes occur in groups as if arising as colonies from budding or in some peculiar way associated with one another.

While such aggregates are well shown in nature on leaves of *Potamogeton* and *Elodea* they can be better illustrated from the groups formed in aquaria on paper, glass and the surface film of the water.

In Fig. 3 is represented a fine example of an aggregate formed in the following way: the *Elodea* from the river was placed in an open dish with opaque sides and motile *Folliculinas* swarmed out, moved toward the light above and came to rest upon floating objects. In this particular case a floating coverglass of 18 mm.

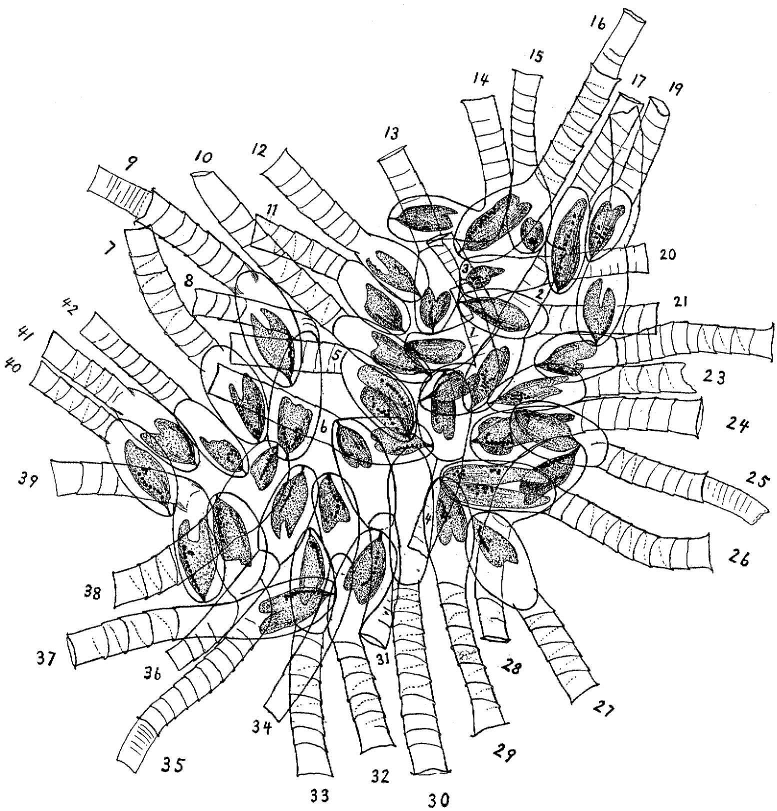

FIG. 3. View from above of a group of 42 that are attached to a floating cover glass with the sacs fastened toward the observer and the tubes reaching down away from him. With the exception of 1, 2, 4, 5, 6, all radiate out around the periphery of the group and most are attached in one plane. Camera lucida from preserved specimens. $\times$ 44.

side received this one aggregate of *Folliculinas* and no other individuals at all. That is for some reason all the *Folliculinas* that came to this area of 324 sq. mm. and settled down, made tubes in one region of only about one sq. mm. very near the center

of the entire glass. In other cases such settlements are made at the edges, or here and there over the glass. Aggregation into groups is the general rule.

It will be noted that the 42 individuals of this aggregate are most all in one plane, spread out over the glass and attached to the glass so that the flat side of the sac, see Fig. 1, is fast to the glass while the tube of the case rises up away from the glass at an angle of nearly 45 degrees, Fig. 1. Fig. 3 was drawn with the camera from the upper side of the cover glass to which the cases were attached below, so that the tubes pass downward from the level of the sacs. While most of the sacs stand side by side in one plane, some overlap and some are fastened deeper down in the water on top of the general stratum of sacs. It is noteworthy that the tubes of all the cases radiate outward, with few exceptions.

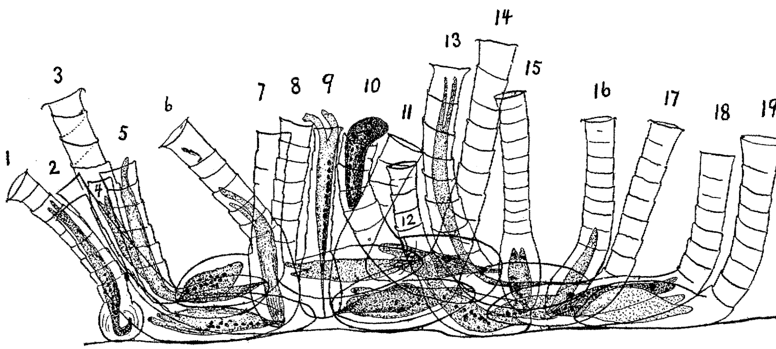

FIG. 4. Side view of a group of nineteen individual *Folliculinas* that made these cases on the edge of a cover glass floating on water. While most of the sacs stand in a row some are behind others and some are across the general trend. A few lie piled upon the others. One, No. 10, is in process of escape from the tube. Camera ucida, preserved material. × 55.

Most of these forty-two are generally alike in size and structure of cases, and with the spirals in the same direction. The animals themselves are but poorly shown, since they were distorted by treatment with too much cocaine in attempting anesthesia; but they show the moniliform nuclei and the stalks of attachment to the bases of the sacs.

Exceptional tubes similar to those of Wrights' specimens are shown in Nos. 9, 16, 25, and 35 in which a second story has been

built out from the old mouth of the tube, and this added extension has but imperfect spiral ridges.

Another aggregate of only nineteen individuals is shown in Fig. 4, as seen from the side. This was formed in the same way but on the edge of another floating cover glass. The continuous line they are attached to is the continuous film they secreted and which was pulled off from the glass with all the cases in making this preparation. Though the thickness of the cover glass was but 150 μ. some sacs are placed back of one another on the end of the glass and so are partly hid by those in front, in the sketch.

Others, however, as 6 and 8, are not fast to the glass but are built up on top of other cases. Here again the cases are densely crowded together with very little space not occupied and the tubes radiate outward from the group as a whole, though in this case the group has the form of a linear aggregate.

In this preparation the animals are better preserved; No. 9 is about to expand outside of its tube; No. 13 is much elongated while in No. 10 is one caught in the act of escaping from its tube to swim away as the motile form without the characteristic long arms of the tube dwellers.

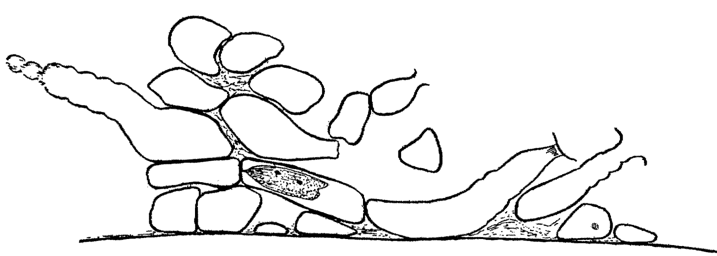

FIG. 5. Section, 6 μ thick, of a group formed on surface of water, to show that not all are on one plane but some are piled on top of others. All are held together by or attached to, a common secretion. Camera lucida $\times$ 66.

That the cases of *Folliculina* are not only made in contact with the foreign substance but also in contact with other tubes and above the general level is shown in actual sections of such aggregates. Thus in the 6 μ section, Fig. 5, several cases are seen piled up on top of others that rest on the common basis which was a pellicle formed by the *Folliculina* on the surface of the water. Between some of these cases not in close contact

there is a mass of coagulum that binds them all together to some extent.

The material binding the cases together, seen as a film in Figs. 4 and 5, is so tenuous and transparent as to easily escape notice, but when the aggregates form on the surface of the water it is this common basis that enables one to pick up the aggregates as one mass and when the leaves of *Elodea* die and macerate, it is easy to obtain innumerable aggregates falling off but each firmly bound into one unit by the film that underlies all the cases.

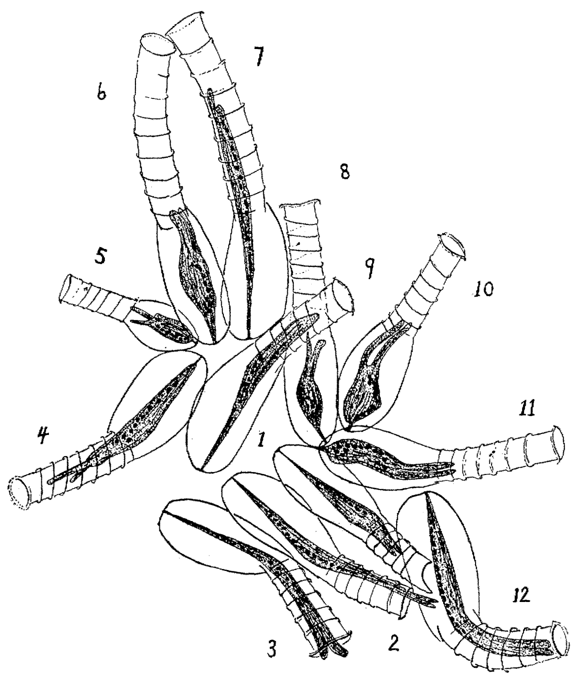

FIG. 6. View from above of a small group of twelve formed on surface of water. Camera lucida × 55.

Even such a loose aggregate as that in Fig. 6, formed on the surface of water is really bound into one mass that may hold together through all the processes necessary for section cutting, though in face view the film is not seen. This last figure well illustrates the tendency of the *Folliculina* to build tubes in a radiating group and to fill in most of the intervals in an irregular way.

Observations upon free swimmers and those making cases throw light upon the above groupings of the cases. The factor of importance in addition to the responses to light and to foreign objects seems to be the secretions put out by the free swimmers.

That the swimming forms may put out considerable amounts of invisible and adhesive secretions was shown in various indirect ways. Thus when india ink is rubbed up with the water in which the *Folliculina* swims, the presence of a secretion following the swimmer is evident. On the addition of granular carmine, swimmers were seen followed by long strings of granules and some included diatoms trailing behind the *Folliculina* four times its greatest length.

Again when the swimmers press upward into drops of water spreading up onto the edges of a procelain dish they get into water films of great thinness and here there seems a distinct film of bluish green material which remains on the porcelain after the *Folliculinas* have been removed.

That this secreted substance must be very adhesive is to be gathered from several cases in which a motile form came into contact with the larva of some aquatic insect, apparently dipterous. These larvæ are relatively large so that the *Folliculina* glides up and down and around the larva, whose diameter is much greater than the length of the *Folliculina*. The tendency of the *Folliculina* to remain attached to the surface of the larva while gliding all over it was most pronounced and its adhesion to it very strong, for the insect violently coiled in figures of eight and struggled with its legs without dislodging the *Folliculina*. When after many minutes the *Folliculina* left the insect a strand of slime was seen stretched between; presently when the insect came near but not into contact with the *Folliculina* they stuck together and the gliding over the surface was resumed.

Some motile forms about to make cases in the film of water rising on the edge of a watch glass were bound together by fine threads of slightly greenish material. Observations of motile forms about to settle down and secrete the case, shows that they come to rest gradually, moving in ever smaller areas and often, as it were, skating about on the posterior end and then flattening the whole body against the substratum. One may become at-

tached by the posterior end and feel about like a leech and later swim off in a short arc to settle again. When carmine grains were added to the water it was evident that the animal was attached by the posterior end to an invisible film or raft of secretion that formed a pellicle over the surface of the water and extended out irregularly in all directions to a distance of one to four times the length of the *Folliculina*.

These thin pellicles that moved on the water with the contractions of the *Folliculina* were probably made by the individual animals from secretions of an adhesive nature and if other *Folliculina* had come near they might have had their motions influenced by this secretion so that they would have tended to settle down in the same neighborhood and so on till many individuals might become crowded together in one region and have all made their cases together as seen in Figs. 6, 3, and 4.

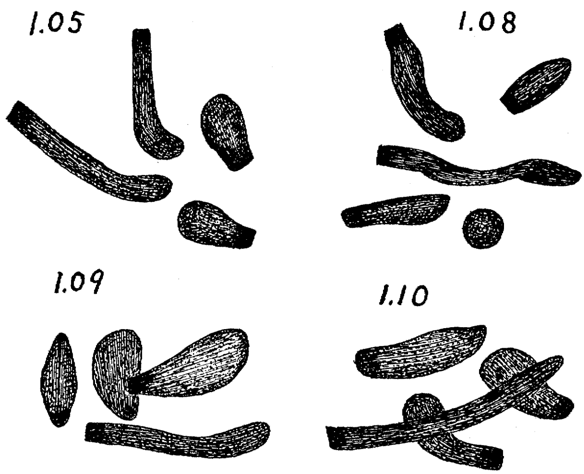

FIG. 7. Sketch of four successive groupings of motile forms that are settling down to form cases. Drawn at intervals of a few minutes from 1.05 to 1.10 p. m.

That several active *Folliculina* may be influenced to act together in one region is shown by the observations illustrated in Fig. 7. Here four motile forms attracted to the same spot remained for a long time with rapid changes of form and position within the group but did not move away from one another's company. Sketched at 1.05 o'clock, the four were radiating

away from one another, at 1.08 a fifth animal approached but soon left the group; at 1.09, three were markedly flattened as indicated by the apparent increase in size, while at 1.10, they had altered positions and shapes. Such oscillations as shown in these five minutes might ultimately result in adjustments of many as in Fig. 6. The common factor that restricts the motions to small areas may well be the secreted film that limits motions by its adhesiveness. The secretion of the first that put out material in the substratum might delay the swimming of another that came near by, lessening the effectiveness of its cilia, mechanically; but other more complex reactions of the second to the secretions of the first may be imagined.

While the secretions made by those settling down on foreign bodies may somehow determine that other *Folliculina* crowd to the same region, special observations and experiments are needed to determine why the cases have the radial arrangement shown in Figs. 3, 4, 6. It is noteworthy that the cases lie either side by side as 1, 2, 3, Fig. 6, or else are so placed that their long axes radiate roughly from some central region. In the prevailing radial arrangements the anterior ends of individuals do not face one another but face away from one another at opposite poles of the radii of the group. The behavior of the motile forms indicated in Fig. 7, suggests a long series of trials or changes of position leading up to the final position assumed when the cases are made, but these animals did not make their cases for several hours and that seems so abnormal that one cannot rely on these specimens as showing the usual mode of settling down though we make use of them as evidence that they are held in one region by something which may well be the secretions.

Possibly careful study would show that the currents set up by the cilia would tend to passively drive the animals into the radial position when the posterior end of each is held somewhat passive by the abundant secretion at that end; but on the other hand some of the groupings, and especially the arrangements of *Folliculinas* that settle down on top of a group that has already made cases suggest that each animal is capable of responding to the presence of others in some complex way, so as to avoid facing another, and so as to fit itself into vacancies amidst a group or to lie parallel to others.

There remain other facts that must wait further observations of elucidation. Why do individuals prevailingly lie along in grooves of the wood? Why do they collect in groups near, but not at the edges of leaves? Why in such cases as Fig. 4, are they confined to the narrow edges of the glass and not extended over the lower face of the glass as well? Why do they not settle on wax or on resinous wood?

Possible many of these facts result simply from the reversing movements on receiving a stimulus; an edge to a groove or an edge to the leaf, or the contact with another animal or with its currents may bring about the same inability to advance over the boundary.

Possible, again, there may be some such problems here as seem to exist in the earthworm in responding to angles and edges. The selection of the middle of the cover glass in Fig. 3, and the settlements along the top of leaves near the edges may involve some resultant of spaces travelled over after stimulation at edges.

While, in a general way, the action of the motile forms toward light, surfaces and in their own secretions may suggest how the cases come to be placed as they are in nature, yet there is probability that the phenomena are much more complex than as yet made out.

Summary.

1. This protozoan occurs in the brackish waters of branches of the Chesapeake Bay in temporary association with the fresh water plants *Elodea* and *Potamogeton*.

2. Its adjustment to the growth of these plants is brought about by the migrations of motile forms that escape from the tubes of the sessile forms.

3. These motile forms respond to light and to solids, and these actions seem to keep them ever settling upon newer parts of the plants during the season.

4. The motile forms add materially to the plankton during the summer.

5. *Folliculina* appears in the early summer and disappears before the plants die down in the autumn, forming part of a

temporary community in which marine forms depend upon the fresh water plants for attachment and existence above the bottom. Presumably these animals migrate in from the salt water and die out every year.

6. The peculiar groupings of *Folliculina* suggesting some common bond may be partly explained as due to the secretions they put forth when about to build their cases.

7. The anatomy of the motile and sessile forms and the mode of formation of the case will be considered elsewhere.

LITERATURE REFERRED TO.

Barrett, C. A.

'70 On a new tube dwelling Stentor. Monthly Mic. Journal, III., 1870.

Claparede and Lachman.

'58 Etudes sur les Infusories et Rhizopodes. Geneva, 1858.

Dons, Carl.

'12 Folliculina Studien, I, II, III. Archiv. f. Protistenkunde, 27, 1912.

'13 IV Tromso Museums Aarshefter, 35–36, 1913.

Entz, Geza.

'84 Uber die Infusorien des Golfs von Neapel. Mitth. Zool. Sta. Napel, V., 1884.

Gruber, A.

'84 Die Protozoa des Hafens von Genoa. Verh. D. Leop. Car. Ak. 46, 1884.

Henneguey, L. F.

'84 Note sur un nouvel infusoire cilie (Ascobius lentus). Archiv. d. Zool. exp. 2, I., II., 1884.

Kent, Saville.

'80–'82 Manual of the Infusoria, London, 1880–1882.

Laachmann, H.

'01–'03 Zur kenntniss des heterotrichen Infusoriengattung Folliculina Lam. Deutsche Sudpolar-Expedition, XII., Zool. IV., 1901–1903.

Leidy, J.

'59 Proc. Acad. Nat. Sci., Philadelphia, 1859.

Möbius, K.

'87 Das Flaschenthierchen, Folliculina ampulla. Abd. Nat. ver. Hamburg, 10, 1887.

Müller, O. F.

'86 Animalcula infusoria fluviatilis et marina. Havniac, 1786.

Ryder, J. A.

'80 On the Occurrence of Freia products, Wright, in the Chesapeake Bay. Am. Nat., 14, 1880.

Schroeder, Olav.

'07 Beitrage zur Kenntniss von *Stentor coeruleus*. Archiv. f. Protistenkunde, 8, 1907.

Wright, Strethill.

Observations on British Protozoa. Q. J. Mic. Sci. 1862, II.

'59 On *Stentor Muelleri* and *S. castanea*. Edinb. New Phil. Journ., 1859.